

Title of the Invention

A novel mutated nerve growth factor beta gene, proteins encoded thereby, and products and methods related thereto.

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Field of the Invention

The invention relates to a gene involved in pain perception, more specifically, the invention relates to a genetic mutation leading to loss of pain perception. The
10 invention also relates to products and methods related to the mutated gene and protein encoded thereby.

Background of the Invention

15 Pain insensitivity can vary from pain syndromes associated with dysfunction in various neurological processes including serious peripheral neuropathy, self-mutilation, and mental retardation to more pure forms of analgesia (Nagasako et al., 2003). In most cases of pain insensitivity, an underlying neuropathy leads to the inability to perceive pain and is denoted insensitivity to pain. In some rare cases of
20 pain insensitivity peripheral neuropathy is absent. These patients have normal peripheral responses to pain but do not seem to perceive the sensation of pain. This syndrome is therefore denoted congenital indifference to pain (Baxter & Okzewski, 1960). The absence of peripheral neuropathy has become a criterion for distinguishing congenital indifference to pain from congenital insensitivity to pain
25 (Dyck et al. 1986). To date, researchers have been unable to identify genes related to pure forms of indifference to pain.

A number of genes have been identified which are linked to peripheral neuropathies with pain insensitivity combined with other neurological dysfunctions like the
30 hereditary sensory and autonomic neuropathies (HSANs). This knowledge has helped advance research and offers treatment efforts for HSAN patients. HSAN is a

group of peripheral neuropathies characterized by loss of pain sensation in combination with other sensory or autonomic abnormalities (Dyck et al. 1986; Thomas, 1993). Five types of HSAN have been identified; one of the most extensively studied being HSAN IV, a congenital insensitivity to pain with anhidrosis which is also called CIPA.

HSAN IV is characterized by profound loss of pain sensitivity leading to injuries, self-mutilation and osteomyelitis, anhidrosis and mental retardation (Rosemberg, 1994). Point mutations in the nerve growth factor receptor gene *TRKA* have been identified in HSAN IV patients, implicating the NGF/TRKA pathway in the pathogenesis of HSAN IV (Indo et al. 1996, Indo 2001). Another HSAN, HSAN I, is caused by a mutation in the *SPTLC1* gene (Dawkins et al. 2001) while HSAN III is associated with a mutation in the *IKBKAP* gene (Anderson et al. 2001; Slaugenhaupt et al. 2001). While these early findings have furthered researchers understanding of complex genomic and neurogenic processes and greatly benefited patients, more work remains to be done in elucidating genetic markers for other neurologic diseases. The identification of additional genes that separate pain pathways from other neurological functions will be important for the advancement of disease understanding. Further, this knowledge could aid in the development of therapies where peripheral pain relief is desired but without effects on other neurological functions.

Another gene of interest for researchers in this area is the nerve growth-factor beta (NGFB) gene. It is a 120 amino acid polypeptide homodimeric protein that acts through specific receptors on sensory and sympathetic neurons to support neuronal survival, promote neurite outgrowth, and enhance neurochemical differentiation. Some portions of the entire gene have been sequenced, see, for example, G. Borsani et al., cDNA Sequence of Human β -NGF, Nucleic Acids Research, Vol. 18, No. 13. Probes to the gene have also been identified; see, for example, Elduque et al., An *EcoRI* polymorphism at the beta-nerve growth factor (NGFB) locus in cattle, Animal Genetics, Vol. 28 (1997) p 322. NGF is one of the most potent growth

factors for cholinergic neurons and has been identified as a promising candidate for treating Alzheimer's disease. In clinical trials for the treatment of Alzheimer's disease, induction of pain has been the major adverse event (Eriksdotter Jonhagen et al. 1998). Despite the contributions of many research teams, much remains
5 unknown about the NGFB gene and its' role in possible disease states.

Summary of the Invention

Therefore, it is an object of the present invention to provide genomic data related to
10 neurological conditions. It is a further object of the present invention to disclose a point mutation within the NGF which correlates to severe pain perception loss disease.

A further object of the invention is to provide an isolated nucleotide sequence
15 according to SEQ ID NO:3, a functional fragment thereof, or a sequence that hybridizes thereto or an isolated polypeptide sequence according to SEQ ID NO:4 or a functional fragment thereof. These objects of the invention may be utilized in plasmids, vectors, or host cells. Such a vector may be a virus vector, such as a DNA virus vector, for example, adeno-associated virus, adenovirus, or herpesvirus, or
20 possibly a retroviral vector such as MoMLV, HIV-1, or SIV. Exemplary host cells include eukaryotic cells, COS cells, prokaryotic cells, 293EBNA cells, and insect cells. Host cells transfected with vectors according to the present invention may express mutated NGFB protein.

25 A further object of the present invention is to provide a pharmaceutical composition which comprises at least one functional fragment of a nucleotide according to SEQ ID NO:1 or a peptide according to SEQ ID NO:2, the pharmaceutical composition being effective for the treatment of a gene disorder marked by the presence of a mutation at a position corresponding to position 298 of SEQ ID NO:3.

Yet another object of the present invention is to supply a molecular probe for the indication a genetic defect. The molecular probe comprises a nucleotide sequence according to SEQ ID NO:3 or a sequence which hybridises to that sequence under stringent conditions and a label for detecting the presence of the sequence. The label
5 could be radioactive.

The invention further provides a method of screening a patient for a genetic defect. The method comprises obtaining a sample of genetic material from the patient and identifying the nucleotide present at a position corresponding to position 298 of
10 SEQ ID NO:3. The patient has a genetic defect if a nucleotide other than cytosine is identified at that position.

A method for detecting the presence of a genetic defect in a biological sample is also provided, where the method comprises contacting the biological sample with a
15 nucleic acid molecule comprising a compliment to SEQ ID NO:3 as a probe in a nucleic acid hybridization assay and detecting whether the nucleic acid molecule has undergone hybridization. Hybridization indicates the presence of a genetic defect in the biological sample.

20 A further object of the invention is to provide a transgenic animal with a modified nucleotide at a position corresponding to position 298 of SEQ ID NO:3. That modified nucleotide can be thymine. The animal can be a mammal, such as a rodent. The animal can comprise one or more cells which express a sequence according to SEQ ID NO:3.

25 Yet another object of the present invention is to provide a method for evaluating the ability of a potential therapy to treat or cure a genetic disorder, which comprises administering the potential therapy to a transgenic animal according to the present invention and evaluating a pain response in said animal. An improved pain response
30 in the animal as compared to untreated similarly-situated transgenic animals indicates that the potential therapy is able to treat or cure a genetic disorder.

The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic
5 of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner
10 similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine. "Amino acid analogs" refers to compounds that have the same basic chemical structure as a naturally occurring amino acid. Such analogs have modified R groups
15 (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid. Amino acids may be referred to herein by either
20 their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

25 The term "conservative modifications" or "conservatively modified variants" as used herein applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences; conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially
30 identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the

codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence with respect to the expression product, but not with respect to actual probe sequences.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

The phrase "stringent hybridization conditions" as used herein refers to conditions under which sequences will hybridize. Stringent conditions are sequence-dependent and will be different in different circumstances. Skilled workers have access to significant amounts of descriptive material detailing reaction conditions that are appropriate for a given sequence. For example, Innis et al. (1990) PCR Protocols, A Guide to Methods and Applications, Academic Press, Inc. N.Y.

The term "drug" as used herein, refers to any medicinal substance used in humans or other animals. Encompassed within this definition are compound analogs, naturally occurring, synthetic and recombinant pharmaceuticals, hormones, neurotransmitters, and the like.

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Brief Description of the Drawing Figures

Figure 1 shows a pedigree of families with pain insensitivity as described below, where squares represent males, circles represent females, solid symbols represent affected individuals, dots indicate obligate carriers and filled bars indicate the disease haplotype;

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Figure 2 shows specific sequence analyses of the NGFB gene, wherein the arrow indicates the mutation at position 661;

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Figure 3 shows the structure of the NGF gene with the alternative amino acid in position 100 highlighted; and

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Figure 4 shows sequence alignment of NGF from different species as well as of human neurotrophins showing the conservation of the mutated amino acid in position 100 of the mature NGF protein.

Detailed Description

While particular embodiments of the present invention have been illustrated and described, it would be obvious to those skilled in the art that various other changes and modifications can be made without departing from the spirit and scope of the invention. It is therefore intended to cover in the appended claims all such changes and modifications that are within the scope of this invention. Materials, the synthesis of which are not specifically described, are either commercially available or can be prepared using methods well known to those of skill in the art. Except as

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otherwise noted, all amounts including quantities, percentages, portions, and proportions, are understood to be modified by the word "about", and amounts are not intended to indicate significant digits. Except as otherwise noted, the articles "a", "an", and "the" mean "one or more". All documents cited are, in relevant part,
5 incorporated herein by reference; the citation of any document is not to be construed as an admission that it is prior art with respect to the present invention

Example 1: Mapping of the locus for pain insensitivity

10 The mapping of the locus for pain insensitivity was performed using data from a single family. The family exhibits three severe cases and at least 10 cases with various degrees of the pain insensitivity. Out of the 13 patients, 6 cases have been investigated with respect to orthopedic, neurological and neurophysiologic status. The clinical evaluation revealed three severely affected individuals with onset in
15 early childhood. The other cases had a milder phenotype. The patients suffer from a loss of deep pain perception that leads to destroyed joints in childhood or, in milder forms, in middle age. Most other neurological functions appear to be intact or only slightly affected, including autonomic responses. Anhidrosis as well as mental retardation is absent in this family. The inheritance pattern for the three severe cases
20 is consistent with an autosomal recessive transmission of the disease. Extensive inbreeding and the fact that the parents of the severe cases are related support this inheritance pattern (See Fig. 1). The number of possible cases present in the family, however, suggested a gene dosage effect resulting in less pronounced symptoms in heterozygote individuals. Due to the difficulty of assigning a correct status for all
25 but the three severe cases, only the three severe cases were used in the genetic analysis.

The three severely affected individuals were screened for shared homozygosity regions based on the hypothesis of a recessive inheritance of the severe form of the
30 disease. The three severely affected individuals, together with their parents and healthy siblings when available, were used in the analyzes. For this, Genomic DNA

from all individuals were prepared from whole blood using standard salt methods and analyzed using an ABI panel with an average distance of 10cM (ABI PRISM Linkage Mapping Set 10 cM version 2.5 (Applied Biosystems, Foster City, CA)). The primers were amplified according to manufacturer's instructions, utilizing
5 multiplex PCR reactions. PCR products were resolved through 36cm capillary arrays using POP-4 polymer on an AB 3100 DNA sequencer. Genotypes were analyzed using AB Genescan 2.1 and GeneMapper 2.0 (Applied Biosystems, Foster City, CA). The genotypes for each individual were ordered into whole-chromosome haplotypes and regions of homozygosity were assessed visually using Cyrillic 2.1
10 (Exeter Software, NY USA). The genome-wide screen revealed a common haplotype, on chromosome 1q12, for which all three severely affected individuals were homozygous (See Fig. 1). The disease haplotype was also observed in unaffected or mildly affected family members, but in heterozygote form only. No other marker loci tested fulfilled the criteria of a recessive gene identical by descent.
15 The disease gene could be restricted to an 8.7 Mb region, close to the centromere, flanked by the markers D1S2809 and D1S185.

Example 2: Identification of the causative gene and mutation

20 The disease critical region contained 66 genes and a number of hypothetical transcripts according to the Celera database. The disease critical region was scrutinized for functional candidate genes. A number of candidate genes were identified in the disease critical region. Out of these, *NGFB* constituted one of the more plausible candidate genes and was chosen for mutational analysis. The *NGFB*
25 gene was analysed by direct sequencing of all exon and exon-intron boundaries. Sequences for exons and exon-intron junctions of candidate genes were retrieved from NCBI's Entrez database and primers were designed using Whitehead's Primer3 program. PCR products were separated on agarose gels and purified using spin columns (Bio-Rad, Hercules, CA). Sequencing was performed using the BigDye 3.0
30 kit according to manufacturer's instructions (Applied Biosystems). Labeled products were resolved through 36cm capillary arrays using POP-4 polymer on an

ABI 3100 DNA sequencer and analyzed using the Sequencing Analysis 3.7 software (Applied Biosystems, Foster City, CA).

The sequencing analysis of *NGFB* revealed a point mutation in exon 3 of the gene specific for the disease haplotype, as shown in Fig. 2, where the affected individual, #8, is homozygous for the mutation, his unaffected parent, #6, is heterozygous and an unaffected relative, #3, is homozygous for the wild type allele. The mutation (661C→T) changes a basic arginine to a non-polar tryptophan at position 211 in the NGFB polypeptide corresponding to amino acid 100 in the mature protein (See Fig. 3). The substituted amino acid is located in a region of the protein that is highly conserved between different neurotrophins as well as NGFs of different species; see the sequence comparison in Fig. 3. One hundred control individuals were screened for the mutation in addition to spouses and unaffected members of the family. The mutation was only detected in the family members with the disease-associated haplotype, proving that the identified mutation in *NGFB* is the cause of the novel loss of pain perception disease in this family, and possibly in many others.

The wild type nucleotide and amino acid sequences are shown in SEQ ID NO.s 1 and 2, respectively. The nucleotide and amino acid sequences with the NGF mutation described herein are given in SEQ ID NO.s 3 and 4, respectively. Conservative modifications could be performed on the sequences described herein and still result in products or methods that fall within the spirit and scope of the appended claims. Similarly, the production of sequences that hybridize to claimed sequences under stringent hybridization conditions are envisioned in the present invention.

Example 3: Characterization of mutation

The phenotype of the NGF mutation described herein was compared to that resulting from the TRKA mutations associated with HSAN IV. A less severe phenotype was documented in the NGF mutation. The fact that a less severe effect is observed for the NGF mutation than mutations characterized in the TRKA receptor shows that the

NGF mutation does not completely abolish the ability of NGF to bind and activate TRKA, or, alternatively, it affects the interaction with the low-affinity receptor p75 thereby inhibiting p75 promoted NGF activities. To elucidate the cause of the decreased severity and thereby improve understanding of this condition and offer screening and treatment possibilities, the location of the mutation was evaluated. The NGF mutation is located in a position highly conserved between NGF and the related neurotrophins, BDNF, NT 3 and NT 4 (See Fig. 4). These factors all bind to the p75 receptor demonstrating that this region is essential for their common ability to bind and/or activate p75. This decreased binding to and/or activation of p75 inhibits p75 promoted NGF activities and leads to the devastating phenotype observed in affected individuals.

Knock-out mice models have been generated for NGF as well as for the NGF receptors TRKA and p75. Comparisons show that homozygous mice with disruptions in any of the three genes NGF, TRKA, or p75, exhibit a deficit in the nociceptive function (Lee et al. 1992; Crowley et al. 1994; Smeyne et al. 1994). Both the NGF and TRKA knock-out mice show profound cell loss in both sensory and sympathetic ganglia. Furthermore, TRKA mice exhibit a decrease in the cholinergic basal forebrain projections to the hippocampus and cortex, demonstrating that the TRKA signaling pathway plays a crucial role in the development of both the peripheral and the central nervous systems (Smeyne et al. 1994). The overall phenotype of the p75 null mice is less severe than both that of TRKA- and NGF null mice. Both the NGF and TRKA null mice die within a few weeks of birth, while the p75 null mice are viable and fertile. This observed difference in phenotype is supported by the newly-demonstrated understanding that the NGF mutation described herein affects the binding to p75, generating a less severe phenotype than would be expected if the NGF binding to the TRKA receptor was affected.

The NGF mutation described for the first time herein separates the effects of NGF mediating normal development of functions of the central nervous system, such as mental abilities, from effects on the nervous system involved in peripheral pain

pathways. This provides an important tool for researchers who need to separate the different roles of NGF.

Example 4: Transgenic animals

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As noted above, the NGF mutation can be used as a platform for research, such research falling within the scope of this invention. For example, the creation of transgenic research organisms is known in the art. Using the novel sequence mutation described herein, researchers may for the first time generate research
10 organisms that possess this mutation, thereby generating an extremely useful research tool.

The present invention specifically contemplates the creation and use of non-human transgenic animals for analysis and experimentation. Transgenic animals containing
15 mutant or modified genes corresponding to those disclosed herein are therefore also included in the invention. Transgenic animals are genetically modified animals into which recombinant, exogenous or cloned genetic material has been experimentally transferred. Such genetic material is often referred to as a transgene. The nucleic acid sequence of the transgene may be integrated either at a locus of a genome
20 where that particular nucleic acid sequence is not otherwise normally found or at the normal locus for the transgene. The transgene may consist of nucleic acid sequences derived from the genome of the same species or of a different species than the species of the target animal. Exemplary animals include mammals, such as rabbits and rodent species such as rats and mice. Exemplary uses of such a transgenic
25 animal are the study of pain processes, reactions, and pathways and the evaluation of potential therapies for the treatment or cure of the condition related to the genetic defect disclosed herein.

Transgenic animals can be produced by a variety of different methods including
30 transfection, electroporation, microinjection, gene targeting in embryonic stem cells and recombinant viral and retroviral infection as known in the art. The method of

introduction of nucleic acid fragments into recombination competent mammalian cells can be by any method that favors co-transformation of multiple nucleic acid molecules. Detailed procedures for producing transgenic animals are available to one skilled in the art, for example, U.S. Pat. Nos. 5,489,743 and 5,602,307.

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Example 5: Genetic Analysis and Treatment

One method commonly accepted for identifying potential irregularities and/or deformities prior to birth is amniocentesis. This procedure and its' benefits are widely described in the art. It is now becoming more common to test patients for genetic abnormalities after birth as well. As knowledge of mutations grows, these common screening procedures can be modified to include screens for newly-revealed mutations. It is contemplated that the specific point mutation according to the present invention can and will be incorporated into genetic screening tests to diagnose patients suffering from this condition, including patients who have not been born.

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After practitioners are armed with the knowledge of the condition, the present invention contemplates gene therapy approaches to the treatment and cure of the condition caused by the NGF mutation described herein. Gene therapy aims to replace the mutated gene with a corrected version of the gene. Various methods for practicing gene therapy are known in the art, for example, those disclosed in U.S. Patent No. 6,627,615. One such method may be interrelated with the preparation of transformed or transfected cells. For example, the desired gene can be inserted into a commercially available plasmid which is then in turn cloned into a cell. The transfected cell can be cultured and a gene or gene product harvested. Such methods and materials are known in the art.

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Example 6: Drug Screening Methods

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In addition to the use of transgenic organisms expressing the mutated NGF of the present invention for evaluation of potential drug therapies against the undesirable side effects of the novel NGF mutation, cell-based screening methods for evaluating potentially effective drug therapies are also envisioned and encompassed by the invention.

One such example is to compare cells or groups of cells under different conditions. The first group is comprised of cells expressing the wild-type NGF which are evaluated for various parameters such as their ability to stimulate the formation and maintenance of normal neuronal signaling pathways. The second group is comprised of cells expressing the mutated NGF described herein, also evaluated for specific nerve growth-related parameters. The third group of cells is comprised of cells expressing mutated NGF according to the present invention. In addition to the media used for the cells, a potential drug candidate would be added to the third group of cells at the appropriate dosage and duration. This third group of cells would be evaluated for specific nerve growth parameters as well. Data from the first two groups could be retained and re-used without repeating the culturing and evaluation, if desired.

Comparisons between the untreated wild type, untreated mutants and treated mutants could be made and potential therapies could be ranked as more or less likely to produce a desired outcome in a patient expressing the mutated NGF of the present invention or in a patient who has the wild type NGF but nonetheless suffers from similar undesired phenotypic changes as patients with the mutated NGF described herein. Using such screening methods, it is possible to screen for potential drug effects on mutated cells.

Further, many drug screening programs which test libraries of compounds and natural extracts are high throughput assays. These are desirable in order to maximize the number of compounds surveyed in a given period of time. Such assays are performed in cell-free systems, such as may be derived with purified or

semi-purified proteins, and are often preferred as "primary" screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound. Moreover, the effects of cellular toxicity and/or bioavailability of the test compound can be generally ignored in the in vitro system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity with receptor proteins.

Accordingly, in an exemplary screening assay of the present invention, the compound of interest is contacted with a mutated NGF polypeptide which is ordinarily capable of binding or interaction with TRKA or p75. The potential receptor is also in the mixture. Detection and quantification of receptor/NGF complexes provides a means for determining the compound's efficacy at inhibiting (or potentiating) complex formation between the receptor protein and the mutated NGF polypeptide. The efficacy of the compound can be assessed by generating dose response curves from data obtained using various concentrations of the test compound. Moreover, a control assay can also be performed to provide a baseline for comparison. In the control assay, isolated and purified mutated NGF polypeptide is added to a composition containing the receptor protein, and the formation of receptor/NGF complex is quantitated in the absence of the test compound.

Example 7: Specific Disease Application

Furthermore, because NGF constitutes one of the most potent growth factors for cholinergic neurons and because of its' suspected link to Alzheimer's disease, elucidating the identified NGF mutation provides new tools for Alzheimer therapy. Potential new therapies could circumvent the more severe side effects of existing therapy. This benefit to patients and practitioners will only grow as the world population ages and even greater numbers of persons become affected by this disease.

The foregoing description and examples have been set forth merely to illustrate the invention and are not intended to be limiting. Since modifications of the disclosed embodiments incorporating the spirit and substance of the invention may occur to persons skilled in the art, the invention should be construed broadly to include all variations falling within the scope of the appended claims and equivalents thereof.

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Sequence Listing

SEQ ID NO:1

tca tca tcc cat ccc atc ttc cac agg ggc gaa ttc tcg gtg tgt gac 48
 agt gtc agc gtg tgg gtt ggg gat aag acc acc gcc aca gac atc aag 96
 5 ggc aag gag gtg atg gtg ttg gga gag gtg aac att aac aac agt gta 144
 ttc aaa cag tac ttt ttt gag acc aag tgc cgg gac cca aat ccc gtt 192
 gac agc ggg tgc cgg ggc att gac tca aag cac tgg aac tca tat tgt 240
 acc acg act cac acc ttt gtc aag gcg ctg acc atg gat ggc aag cag 288
 gct gcc tgg cgg ttt atc cgg ata gat acg gcc tgt gtg tgt gtg ctc 336
 10 agc agg aag gct gtg aga aga gcc tga 363

SEQ ID NO:2

Ser Ser Ser His Pro Ile Phe His Arg Gly Glu Phe Ser Val Cys Asp
 1 5 10 15
 15 Ser Val Ser Val Trp Val Gly Asp Lys Thr Thr Ala Thr Asp Ile Lys
 20 25 30
 Gly Lys Glu Val Met Val Leu Gly Glu Val Asn Ile Asn Asn Ser Val
 35 40 45
 Phe Lys Gln Tyr Phe Phe Glu Thr Lys Cys Arg Asp Pro Asn Pro Val
 20 50 55 60
 Asp Ser Gly Cys Arg Gly Ile Asp Ser Lys His Trp Asn Ser Tyr Cys
 65 70 75 80
 Thr Thr Thr His Thr Phe Val Lys Ala Leu Thr Met Asp Gly Lys Gln
 85 90 95
 25 Ala Ala Trp Arg Phe Ile Arg Ile Asp Thr Ala Cys Val Cys Val Leu
 100 105 110
 Ser Arg Lys Ala Val Arg Arg Ala
 115 120

30 SEQ ID NO:3

tca tca tcc cat ccc atc ttc cac agg ggc gaa ttc tcg gtg tgt gac 48
 agt gtc agc gtg tgg gtt ggg gat aag acc acc gcc aca gac atc aag 96
 ggc aag gag gtg atg gtg ttg gga gag gtg aac att aac aac agt gta 144
 ttc aaa cag tac ttt ttt gag acc aag tgc cgg gac cca aat ccc gtt 192
 35 gac agc ggg tgc cgg ggc att gac tca aag cac tgg aac tca tat tgt 240
 acc acg act cac acc ttt gtc aag gcg ctg acc atg gat ggc aag cag 288
 gct gcc tgg tgg ttt atc cgg ata gat acg gcc tgt gtg tgt gtg ctc 336
 agc agg aag gct gtg aga aga gcc tga 363

40 SEQ ID NO:4

Ser Ser Ser His Pro Ile Phe His Arg Gly Glu Phe Ser Val Cys Asp

1 5 10 15
Ser Val Ser Val Trp Val Gly Asp Lys Thr Thr Ala Thr Asp Ile Lys
 20 25 30
Gly Lys Glu Val Met Val Leu Gly Glu Val Asn Ile Asn Asn Ser Val
5 35 40 45
Phe Lys Gln Tyr Phe Phe Glu Thr Lys Cys Arg Asp Pro Asn Pro Val
 50 55 60
Asp Ser Gly Cys Arg Gly Ile Asp Ser Lys His Trp Asn Ser Tyr Cys
65 70 75 80
10 Thr Thr Thr His Thr Phe Val Lys Ala Leu Thr Met Asp Gly Lys Gln
 85 90 95
Ala Ala Trp Trp Phe Ile Arg Ile Asp Thr Ala Cys Val Cys Val Leu
 100 105 110
Ser Arg Lys Ala Val Arg Arg Ala
15 115 120